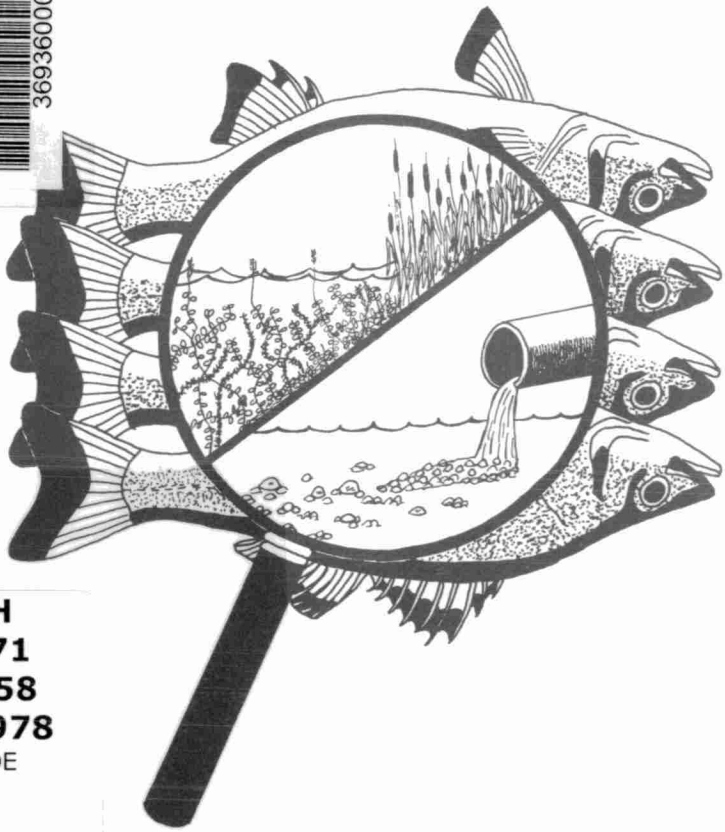
INVESTIGATING FISH KILLS:

A Guide to Field Procedures



Ontario
Ministry of the Environment
Hon. George R. McCague, Minister
K. H. Sharpe, Deputy Minister

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G.L. Beggs
Limnology and Toxicity Section
Water Resources Branch
Ministry of the Environment
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SUMMARY

1. Request the assistance of local witnesses to act as guides for the investigation and use their services to assist in determining the physical extent, the duration, and suspected causes of the kill. Local observers are invaluable sources of information about unusual events in the vicinity of the kill, weather immediately prior to the kill, and possible point sources of pollution.

2. Estimate the total numbers of fish, the species and the size ranges affected. Record all observations of other dead wildlife in the kill area.

3. Record the physical properties of the affected water with particular reference to unusual colour, clarity, odour, aquatic plant growth or flow.

4. Measure temperature and oxygen levels at several depths in the water and determine if the water pH falls within the expected range.

5. Collect water samples in duplicate for all chemical analyses. Choose the chemical analyses based on the suspected causes of the kill and refer to Appendix B for required volumes, preservation techniques, and containers. Sample in the affected area and upstream and downstream of the kill, when possible. Samples should be collected from possible pollution sources or waste outfalls, and from concentrated pesticides applied on or near the affected waters.

   N.B. Steps 6-8 are followed only when dictated by the circumstances of the kill (see relevant sections in the text).

6. Fish collected for any examination should represent the dominant species and size classes.

   A. For histopathological examination, preserve all fish or tissue samples in 10% formalin.

   B. For isolation of bacteria, viruses or identification of parasites, wrap specimens in wax paper, place in plastic bags and quickly freeze.

C. To analyze fish for metal residues, freeze individual specimens in labelled plastic bags.

D. For determination of pesticide or organic tissue residues, wrap individual specimens in hexane-washed aluminum foil and freeze.

For detailed instructions refer to the Biological Sampling section and Appendices C and D.

7. For phytoplankton identification take duplicate one litre water samples from the euphotic zone (twice the Secchi disc). Fill one bottle to about .75 litre and fix the other sample with Lugol's iodine solution.

8. For bioassay testing, duplicate 20 litre water samples should be collected from the affected water body or waste outfall and, ideally, both upstream and downstream of these sites. If buckets are unavailable collect a minimum of two 1 litre water samples in standard litre glass bottles. Do not chemically preserve the bioassay sample. Always collect water samples for chemical analyses from the same sites as the bioassay samples.

9. Particularly in the case of possible legal action, all chemical and biological samples should be sealed with labelled wax or masking tape. In addition to sample submission sheets, the investigator should keep a complete record of sampling locations, time, date, sample numbers, preservatives etc. in a field notebook.

10. After completion of all facets of a fish kill investigation, the investigator should prepare a concise report including all pertinent observations and measurements. Copies of the report should be made available to the parties involved. All field notes, laboratory analyses, and bioassay bench sheets should be retained with a copy of the final report in case they are required for legal action or future investigations.

N.B. <u>The order of a fish kill investigation should reflect the relative importance and urgency of each piece of evidence</u>.

INTRODUCTION

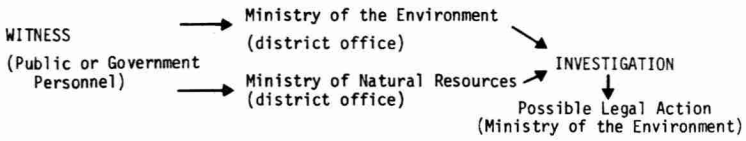
Extensive fish mortality can result from a variety of causes of either natural or man-made origin. Although most reported fish kills occur during the summer months, winter fish kills are not uncommon. Fish kills can occur in natural systems, at fish hatcheries, fish farms and in laboratory holding facilities.

Natural fish kills can be attributed to fluctuations in water quality caused by phenomena such as extreme temperature changes, oxygen depletion resulting from biological decomposition, algal toxins, high water turbidity and gas supersaturation. Fish mortality may also be caused by bacterial, viral and parasitic epidemics or may be related to spawning.

Human-related fish kills are generally attributable to the release of heated effluents or toxic chemicals from industrial, agricultural, municipal, or other sources. Additionally, the use of pesticides in agriculture and aquatic weed control has caused some extensive mortalities. Fish kills may also result from the manipulation of water levels or flow. Some of the more frequent natural and human-related fish kills have been briefly summarized in Appendix A.

Fish kill investigations are conducted by regional or district personnel of the Ministry of the Environment or the Ministry of Natural Resources in order to identify causes, suggest remedial measures, establish preventative measures and collect evidence for possible litigation procedures. Speed is of utmost importance in the preliminary stages of a successful investigation. As fish decompose rapidly, particularly in warm weather, evidence of the kill or the cause of death may be unidentifiable within hours. Similarly, in river systems, toxic chemicals and fish corpses may travel downstream rendering it difficult to discern the cause of the kill.

The investigation of a fish kill can be expedited by an effective reporting procedure:



The information required from the witness includes: witness's name and address, exact location of the kill, date and time noted, duration of kill, extent of kill and suspected causes. Observers should be requested to remain at the scene and to act as guides for the investigation.

INVESTIGATION PROCEDURE

After notification of a fish kill the investigation should be conducted by two experienced investigators. A team of two investigators ensures a safe and speedy investigation. The results of some investigations may ultimately be used as legal evidence. Thus, all investigations should be conducted according to the standards required for legally sound evidence.

Equipment

A fish kill investigation kit should be prepared and kept maintained by all offices that may be involved with investigations. Following is a list of equipment necessary for effective investigations:

- field book, pencils, pens
- Oxygen meter (calibrated) or Hach Kit
- temperature probe (calibrated)
- pH meter (calibrated) or pH indicator strips
- lead line for depth measurements
- long-handled dip net
- water sample bottles, labels and sample preservatives (refer to "A Guide to the Collection and Submission of Samples for Laboratory Analysis" for details).

  Minimum requirements include:

  10 - 1 litre glass bottles for routine water quality parameters
  10 - acid-washed plastic bottles for metal analyses
  10 - 1 litre glass bottles for pesticide scans
- plastic bags and glass sample jars for biological specimens or sediment samples
- cooler and freezer pack (or pick up ice)
- lockable sample box, masking tape for seals and labels
- 2 - 20 litre lidded buckets for bioassay water samples
- tape measure, corks, stopwatch
- camera and film

Several other pieces of equipment may be necessary depending on the location, extent, and type of kill:

- secchi disc
- dredge
- Van Dorn water sampler
- sieves and forceps
- seine net
- Lugol's iodine solution-preservative for phytoplankton samples.
- 10% formalin-preservative for fish and bottom fauna (1 ℓ minimum)
- hexane-washed aluminum foil (to wrap specimens for pesticide or PCB analysis)
- hip or chest waders, life preservers, boat and motor, paddles

Preliminary Information

On arrival at the site the witness and/or other local people should be retained as guides. All pertinent information concerning the kill should be recorded in a field book. The order of investigation should reflect the relative importance and urgency of each piece of evidence. For example, in flowing water systems when a contaminant problem is suspected, chemical sampling should be high on the list of priorities. Mapping of the site and accompanying photographs could be done towards the end of the investigation.

With the assistance of local observers the investigators must determine when the fish kill began and ended. The number of fish mortalities should be estimated as accurately as possible. When dealing with extensive kills, counts may be impractical but a total estimate can be made by extrapolating the count from a small subsection of the area. The species affected should be identified by common and scientific names and the relative proportion of each species recorded. If the size of fish affected appears to be non-random, a record of the predominant size range should be made.

Observations of other dead wildlife, mammals, birds, invertebrates should also be recorded.

Reporting any unusual events occurring in the vicinity of the kill may assist in assessing the cause. Some examples of practices that may adversely affect water quality include: agricultural spraying of pesticides, runoff from fertilized fields, road construction, road salting, industrial breakdowns, chemical spills, application of aquatic pesticides, and water level fluctuations. Relevant information supplied by local observers should be recorded and the affidavit signed by the witness.

Physical Observations

Descriptions of the physical properties of the affected waters often provide information essential for defining the cause of a fish kill. Important characteristics to examine include the colour, clarity and odour of the water, aquatic growths such as algae, or excessive macrophytes. The limits of the affected area should be ascertained and rigorously recorded with reference to legal and political land divisions. A map of the fish kill area including its limits, sampling locations, waste outfalls and pertinent land use patterns is an important component of physical observations. Delineation of boundaries may be difficult in flowing water where dead fish and chemical evidence may be carried downstream. Examination of tributaries entering the kill vicinity may identify the problem's source or a refuge for fish that escaped the kill. Accurate descriptions can be facilitated by photographs of the site, including identifiable landmarks.

Observations on prevailing weather conditions at the time of and 2-3 days prior to the kill should be described and confirmed from weather bureau sources after the on-site investigation. Of particular note are air temperatures, precipitation and wind direction.

River and stream flows and inflows in the case of ponds and lakes, should be gauged and recorded. Of particular note are instances of high flow, intermittent flow or stagnant water conditions. Flow can be simply estimated using a cork and stopwatch and measuring average width and average depth of the water course:

FLOW	=	mean width	x	mean depth	x	speed of cork
($meters^3/sec.$)		(meters)		(meters)		(meters/second)

Temperature fluctuations can contribute to or directly cause fish mortalities. Temperatures should be recorded at several locations and at a series of depths in the system. When lethal temperatures are suspected a diurnal temperature profile may be necessary to ensure a record of temperature maxima.

Chemical Measurements

Water sampling for chemical parameters should be conducted at several stations in the kill area to determine the extent and to locate

possible point sources of contamination. Pollution sources can be identified by noting the point where dead fish first appeared and/or by information supplied by local people. In flowing water systems, sampling upstream and downstream of the affected area is essential. Additionally, samples should be collected upstream and downstream from all known discharges or possible pollution sources and from all points of confluence in the kill vicinity. In large lakes and rivers, sampling stations should be located on transect lines which originate from an identifiable pollution source. Surface and bottom water samples should be supplemented with samples at regular depth intervals. If the pollutant can no longer be located in the kill area, water samples can be collected in shoreline pools or backeddies where concentrated remnants of the contaminant may remain.

The presence of a waste outfall does not necessarily indicate the cause of a fish kill. Other evidence is necessary and all potential causes should be investigated. Unwarranted assumptions may result in collection of insufficient evidence and an incomplete investigation.

Oxygen levels should be measured with a portable meter or Hach kit to establish that conditions are sufficient to support aquatic life. Measurements from several depths and possibly at various times may be necessary. Investigators may sometimes need to establish a 24-hour oxygen fluctuation cycle to ensure reporting of minimum levels. Water temperature records should coincide in time and location with oxygen profiles.

Water pH is also a variable readily adaptable for field measurements and important as a basis for establishing water quality.

Water sampling:

Water sampled for all chemical analyses should be collected in duplicate. Three times the necessary volume is recommended to enable analytical confirmation or presentation in court as evidence. Beyond routine water quality assessment, the chemical analyses requested should reflect the suspected cause of the kill. For example, in an agricultural area, information from a witness indicating recent local application of a pesticide, or the presence, on site, of a labelled pesticide container, would warrant analysis for a specific compound. A pesticide scan is used only in circumstances in which no clues as to the pertinent compound are available. When pesticides or other toxic compounds are suspected, a sample

of the concentrate should be collected for analysis and comparison with the water analyses.

Water sample bottles are chosen on the basis of the desired analysis. Bottle type and the volume necessary should be confirmed by referring to "A Guide to the Collection and Submission of Samples for Laboratory Analysis" by the Water Quality Section, Laboratory Services Branch. This guide also describes sampling technique, sample preservation and sample submission procedures for water quality parameters, microbiological samples, and sediment samples. Samples obtained without following these guidelines may be impossible to analyze and important evidence lost. Summaries of pertinent information from this guide have been included in Appendix B.

Sampling for legal action:

Sampling for potential legal action requires special care. The investigator must keep a complete record of sampling locations, time, date, bottle numbers, preservative, etc., in addition to sample submission sheets. Samples should be sealed with labels, wax, or labelled masking tape to ensure that it can be proven that they were not tampered with. The sampler must be able to swear that samples were in his possession, or not tampered with until delivery to the analyst. Transportation in a locked truck or trunk of a car meets this requirement. If samples must be shipped, packing boxes should be locked and the keys sent by registered mail to the analyst. To return legal samples, the Toronto Laboratory Stores can provide special locks for which only the main laboratory analysts have keys. These criteria apply for all types of samples taken and all observations recorded.

Biological Sampling - Fish:

Samples of moribund (dying), and freshly-killed fish and other aquatic organisms may provide useful information for several facets of a fish kill investigation. Ideally, healthy fish of the same species from immediately outside the kill area should also be collected for comparison. Although acutely lethal levels of contaminants generally do not cause high tissue accumulation in fish, the cause of death may sometimes be suggested by tissue residue analysis or by pathological and/or histological examination of the corpses. Tissue residue determinations of metals, pesticides, and organic trace contaminants can be done by sections of the Laboratory Services Branch, Ministry of the Environment. However, as the Ministry of the Environment

does not have resident experts in fish necropsy, the identification of fish diseases, parasite, and bacterial infections is generally not feasible. In special circumstances, the resources of experts at Ontario universities may be called upon.

Fish collection for any examination should represent predominant species and size classes. The numbers of fish necessary will be dictated by the type of examination. Generally four or five medium sized specimens from each species or size class will suffice. If fish are very small a larger sample size will be necessary.

Field notes containing all relevant information should be kept for each specimen collected. Pertinent details to report include: location, date, time, method of capture, species, size (length and weight), and sex of each fish. Additionally, a gross description and photographs of physical abnormalities of each fish should be included. Observations on unusual behavioural actions of the fish prior to death may also assist in identification of the cause of death. Scale samples, for age determination of each specimen, should be taken prior to preservation and stored in labelled envelopes. All biological samples should be identified with a sample number on the specimen, in field notes, and on submission forms. A log of the sample condition and handling at each stage of examination should be maintained and the shipping precautions, outlined for chemical samples, followed.

Sampling for histopathological examination:

Fish specimens collected for histopathological examination should be moribund. Best results are obtained if samples are delivered to a pathologist quickly. Photographs of specimens, particularly physical abnormalities, should be taken first and the specimen then placed in the preservative. Although no universal fixative exists, samples are best preserved in 10% formalin (10 mls. commercial formalin in 90 mls. water). The procedure for sample preparation has been outlined in Appendix C.

Sampling for disease or parasite identification:

For isolation of bacteria, viruses or identification of parasites, unhealthy fish should be killed, individually wrapped in wax paper, placed in plastic bags and frozen quickly (Appendix C). For comparison, healthy fish of the same species should be captured from an adjacent area, killed and prepared in the same way as the unhealthy fish.

Sampling for tissue residue analysis:

For tissue residue analysis of fish chronically exposed to a contaminant, a sample of fish muscle from above the lateral line is dissected from a dead specimen and then preserved for analysis (Appendix D). In the case of a fish kill, however, it is unlikely that fish exposed to a lethal concentration of a contaminant, will have accumulated detectable muscle levels. It is sometimes possible, however, to detect contaminant residues in other parts of the body. If tissue residue determinations for metals are necessary, approximately 15 to 20 freshly-killed specimens of each species should be frozen separately in labelled plastic bags. Before submitting samples, D. Russel of the Air Quality Section, Ministry of the Environment, should be contacted (phone 416-248-3023) to arrange the special analyses.

When pesticides or PCB's are implicated in a fish kill and tissue residue determinations required, approximately 5 medium-sized (15 cm.) specimens should be wrapped in hexane or acetone washed aluminum foil prior to freezing. Multiple washing of the foil with hexane or acetone is a necessity. If possible, the investigator should determine the name and/or chemical formulation of the compound in question and, ideally, obtain a sample of the concentrated material to assist the scientist analyzing the tissue samples. An empty pesticide container or contaminated vegetation samples may also prove helpful. To arrange special tissue residue analyses for pesticides, G. Rees of the Pesticides Section, Ministry of the Environment, should be contacted (phone 416-248-3031).

If an organic compound is the suspected cause of a fish kill, special tissue residue analyses can be arranged with Dr. O. Merez of the Organic Trace Contaminants Section, Ministry of the Environment (phone 416-248-3032). Specimens should be wrapped twice in hexane washed aluminum foil and frozen immediately. Five medium-sized (15 cm) fish specimens should provide ample tissue for residue determinations. Any information on the specific compound involved should be supplied with the fish samples to assist the analyst in his determinations. With any contaminant-caused fish kills, duplicate water samples from the site, taken coincidentally with the fish specimens, should be submitted for analysis.

Phytoplankton:

The circumstances of some fish kills may warrant sampling other components of the biota. For example, fish kills may directly or indirectly be related to phytoplankton blooms. To conclusively determine that a phytoplankton bloom, collapse, and subsequent oxygen depletion caused a fish kill, the water should ideally be monitored for algal assemblages prior to the kill. However, as this is not usually feasible, identification of phytoplankton after a kill will determine if bloom-forming species were present and thus provide evidence to negate or support a bloom and collapse theory. Although rarely, fish kills have been associated with diurnal oxygen fluxes due to large phytoplankton or macrophyte populations and have also been attributed to fish toxins produced by algae (Appendix A).

Duplicate water samples for phytoplankton identification should be collected in one liter glass bottles. In shallow well-mixed systems samples can be collected at the surface. A composite sample from the euphotic zone (approximately two times the Secchi disc reading) should be collected in deeper, stratified situations. An air space should be maintained in one bottle (fill to approximately .75 liters) and the other sample should be fixed with Lugol's iodine solution. Lugol's iodine is added to the water until a pale yellow straw colour is reached. Samples should be identified on the bottle label, in field notes, and on sample submission forms, the bottles sealed and delivered to the Plankton Taxonomy Unit, Water Resources Branch, Ministry of the Environment, Resources Road, Rexdale, Ontario. Unpreserved samples collected during the winter months should be kept cold during transportation to the laboratory.

Benthos:

In large water systems many fish may escape a toxicant or corpses may be washed downstream. However, the extent of an affected area can be determined by examining benthic organisms. Benthos are relatively restricted in mobility and are often unable to avoid contact with lethal levels of contaminants. Sampling of benthos in deep waters is best accomplished with

an Ekman dredge or similar tool, and in shallow waters, depending on bottom conditions, by using a dredge or Surber sampler. Obtaining and examining a sample for dead benthos may be time-consuming. Consequently, it should only be employed as an accessory tool when more urgent sampling has been accomplished. Moreover, benthic organisms may be subject to drift in flowing conditions and may be relatively insensitive to water quality conditions which may adversely affect fish or vice versa.

Bioassay Samples :

In some situations it is advantageous to collect large volume water samples from the kill area for bioassay testing. Sampling for bioassay is warranted when legal proceedings may be initiated, to establish lethality of waters in which no biological evidence remains, and to test the lethality of outfalls which may be affecting the kill area. Bioassays may assist fish kill investigations by documenting toxic conditions, identifying sources of contamination, and providing information about the degree of lethality and time factors involved.

Ideally, duplicate 20-litre water samples should be collected from the affected water body or waste outfall. Water upstream of the kill should also be sampled in the same manner. Polyethylene buckets previously detergent washed, rinsed and then preferably acid-washed and rinsed again make the best sample containers. Two 20-litre samples permit replicate laboratory tests of 10 rainbow trout. When buckets are unavailable a minimum of two 1-litre standard glass bottles will enable replicate bioassays employing guppies or daphnia. These 1-litre bottles should also be washed using the method described for the polyethylene containers. Bioassay testing must be backed up with chemical analyses so both types of samples should be collected simultaneously. For advice on the required analyses, the regional industrial abatement officer or the Toxicity Unit staff (Resources Rd., Rexdale) should be contacted.

Bioassay samples should not be preserved chemically. Samples should be capped leaving a minimum air space, labelled, sealed and ideally kept refrigerated (4°C) and locked during transportation and storage.

The Toxicity Laboratory Coordinator or a Toxicity Scientist of the Limnology and Toxicity Section, Water Resources Branch, Ministry of the

Environment (Telephone (416) 248-3011) should be notified about the bioassay sample as soon as possible. At that time, arrangements for transportation of the sample to the laboratory at Resources Road, Rexdale, should be discussed. In many circumstances the toxicity of a sample may diminish with time, necessitating a hasty delivery. If the sample must be shipped, arrangements for pick-up should also be finalized with the Laboratory Coordinator. Samples arriving at Resources Road should first be taken to sample reception in the shipping area and then redirected to the Toxicity Laboratory. Details of sampling, including: date, time, site, suspected causes, and the collector's name, should accompany each sample, preferably on an attached label.

REPORTING

After completion of all facets of a fish kill investigation a report should be prepared and copies made available to all the parties involved. A concise technical writing style is essential for a good report. Reference to the kill location, date, time, and witnesses should be made. Site description can be facilitated by maps and/or relevant photographs. The results of the investigation should be reported, including: the extent of the kill, the numbers and species of fish affected, pertinent physical and chemical measurements, and the results of chemical and biological analyses. Raw data can be tabulated and appended to the report. Conclusions about the cause of the kill should be documented and any pertinent literature references included. If possible, the investigator should suggest remedial and/or preventative measures.

In addition to a copy of the final report, all field notes, laboratory analyses forms, pathological reports, and bioassay bench sheets should be retained in an appropriate file in case they are required for legal actions or future investigations.

ACKNOWLEDGEMENTS

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LITERATURE CITED

1. ALABASTER, J.S. 1969. Survival of fish in 164 herbicides, insecticides fungicides, wetting agents and miscellaneous substances. International Pesticide Control March/April:29-35.

2. BOUCK, G.R. 1973. Total dissolved gases (supersaturation). In Water Quality Criteria 1972. U.S. Environmental Protection Agency, Ecological Research Series. EPA-R3-73-003 March, 1973. Washington, D.C.

3. BRETT, J.R. 1944. Some lethal temperature relations of Algonquin Park fishes. University of Toronto Studies, Biological Series, No. 52. Publications of the Ontario Fisheries Research Laboratory, No. 63. The University of Toronto Press. 49p.

4. BRUNGS, W.A. 1973. Effects of residual chlorine on aquatic life : literature review. J. Water Poll. Contr. Fed. 45(10):2180-2193.

5. BRUNGS, W.A., J.H. McCORMICK, T.W. NEIHEISEL, R.L. SPEHAR, C.E. STEPHAN and G.N. STOKES. 1977. Effects of pollution on freshwater fish. J. Water Pollution Control Federation. 49:1425-1493.

6. CHERRY, D.S., K.L. DICKSON and J. CAIRNS Jr. 1975. Temperature selected and avoided by fish at various acclimation temperatures. J. Fish. Res. Board Can. 32:485-491.

7. COUTANT, C.C. 1977. Compilation of temperature preference data. J. Fish. Res. Board Can. 34(5):739-746.

8. COUTANT, C.C. and S.S. TALMADGE. 1977. Thermal effects. J. Water Pollution Control Federation 49:1369-1425.

9. DAVIS, J.C. 1975. Minimal dissolved oxygen requirements of aquatic life with emphasis on Canadian species: a review. J. Fish. Res. Board Can. 32(12): 2295-2332.

10. DOUDOROFF, P. and D.L SHUMWAY. 1967. Dissolved oxygen criteria for the protection of fish. Trans. Am. Fish Soc. Special Publication No. 4: 13-19.

11. EUROPEAN INLAND FISHERIES ADVISORY COMMITTEE. 1968. Water Quality Criteria for European Freshwater Fish - Report on Water Temperature and Inland Fisheries based mainly on Slavonic literature. EIFAC Technical Paper No. 6. F.A.O. United Nations. 32p.

12. EUROPEAN INLAND FISHERIES ADVISORY COMMISSION. 1970. Water Quality Criteria for European Freshwater Fish. Report on Ammonia and Inland Fisheries. EIFAC Technical Paper No. 11. Food and Agriculture Organization of the United Nations. 12p.

13. EUROPEAN INLAND FISHERIES ADVISORY COMMISSION. 1973. Water Quality Criteria for European Freshwater Fish. Report on Chlorine and Freshwater Fish. EIFAC Technical Paper No. 20. Food and Agriculture Organization of the United Nations. 10p.

14. EUROPEAN INLAND FISHERIES ADVISORY COMMISSION. 1973. Water Quality Criteria for European Freshwater Fish. Report on Dissolved Oxygen and Inland Fisheries. EIFAC Technical Paper No. 19. Food and Agriculture Organization of the United Nations. 10p.

15. GERACI, J.R. and B. HICKS. 1977. Fish Disease Workshop. New England Aquarium. July 10-14, 1977. Unpublished manuscript.

16. GREAT LAKES WATER QUALITY BOARD. Great Lakes Water Quality. Annual Reports of the Water Quality Objectives Subcommittee. 1974-1977.

17. HEATH, A.G. 1977. Toxicity of intermittent chlorination to freshwater fish : influence of temperature and chlorine form. Hydrobiologia 56(1):39-47.

18. HERRICKS, E.E. and A.S. BUIKEMA. 1977. Effects of pollution on freshwater invertebrates. J. Water Pollution Control Federation. 49:1493-1506.

19. HOFFMAN, G.L. 1967. Parasites of North American Freshwater Fishes. University of California Press. Berkley and Los Angeles. 486p.

20. JENSEN, K.W. and E. SNEKVIK. 1972. Low pH levels wipe out salmon and trout populations in southernmost Norway. Ambio 1(6):223-225.

21. JOHNSON, D.W. 1967-68. Pesticides and fishes - A review of selected literature. American Fisheries Society 96-97:398-424.

22. LEIVESTAD, H., G. HENDREY, I.P. MUNIZ and E. SNEKVIK. 1976. Effect of acid precipitation on freshwater organisms. Research Report FR 6/76. Impact of Acid Precipitation on Forest and Freshwater Ecosystems in Norway. May, 1976: 87-111.

23. LEIVESTAD, H. and I.P. MUNIZ. 1976. Fish kill at low pH in a Norwegian river. Nature 259 (5542):391-392.

24. LELAND, H.V., S.N. LUOMA and D.J. WILKES. 1977. Heavy metals and related trace elements. J. Water Pollution Control Federation 49:1340-1369.

25. LIVINGSTON, R.J. 1977. Review of current literature concerning the acute and chronic effects of pesticides on aquatic organisms. CRC Critical Reviews in Environmental Control. November 1977: 325-351.

26. NEBECKER, A.V., G.R. BOUCK and D.G. STEVENS. 1976. Carbon dioxide and oxygen-nitrogen ratios as factors affecting salmon survival in air-supersaturated water. Trans. Am. Fish. Soc. 105(3):425-429.

27. ONTARIO MINISTRY OF THE ENVIRONMENT. 1974. Guidelines and Criteria for Water Quality Management in Ontario. 26p.

28. PICKERING, Q.H. and C. HENDERSON. 1966. The acute toxicity of some heavy metals to different species of warmwater fishes. J. Air Water Pollut. 10:453-463.

29. PICKERING, Q.H. and C. HENDERSON. 1966. The acute toxicity of some pesticides to fish. The Ohio Journal of Science 66(5):508-513.

30. PICKERING, H., C. HENDERSON and A.E. LEMKE. 1962. The toxicity of organic phosphorus insecticides to different species of warmwater fishes. Trans. Am. Fish Soc. 91(2):175-184.

31. PRESCOTT, G.W. 1968. The Algae : A Review. Houghton Mifflin Co., Boston. 436p.

32. REHWOLDT, R.E., E. KELLEY, and M. MAHONEY. 1977. Investigations into the acute toxicity and some chronic effects of selected herbicides and pesticides on several fresh water species. Bulletin of Environmental Contamination and Toxicology. 18(3):361-365.

33. RIBELIN, W.E. and G. MIGAKI (Eds). 1975. The Pathology of Fishes. The University of Wisconsin Press. 1004p.

34. SCHOFIELD, C.L. 1976. Acid precipitation : effects on fish. Ambio 5 (5-6):228-230.

35. SCHWIMMER, M. and D. SCHWIMMER. 1968. Medical aspects of phycology. In Jackson, D.F. (Ed.) Algae, Man and the Environment Syracuse U.P., N.Y., U.S.A. 554 p.

36. SCOTT, W.B. and E.J. CROSSMAN. 1973. Freshwater Fishes of Canada. Bulletin 184. Fisheries Research Board of Canada, Ottawa 1973. 966p.

37. TRACY, H.B. and J.C. BERNHARDT. 1973. Guidelines for Evaluating Fishkill Damages and Computing Fishkill Damage Claims in Washington State. State of Washington. Dept. of Ecology. Technical Report No. 72-10. 29p.

38. U.S. Dept. of the Interior. Division of Technical Support. Federal Water Pollution Control Administration. 1970. Investigating Fish Mortalities. 21p.

39. UNITED STATES ENVIRONMENTAL PROTECTION AGENCY. 1976. Quality Criteria for Water. July, 1976. 256p.

40. Water Quality Section, Laboratory Services Branch. 1977. A Guide to the Collection and Submission of Samples for Laboratory Analysis. Second Edition.

41. WEDMEYER, G.A., F.P. MEYER and L. SMITH. 1976. Disease of Fishes. Book 5: Environmental Stress and Fish Diseases. Snieszko, S.F. and H.R. Axelrod (Eds.). T.F.H. Publications, Inc. Ltd. 192p.

APPENDIX A: Some of the more frequent causes of fish kills and some appropriate references.

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1) Dissolved Oxygen. Reduction in the level of available oxygen has a marked effect on many physiological, biochemical and behavioural processes in fish. Oxygen requirements differ for species and are influenced by season, temperature and activity. Oxygen depletion may result from natural causes: decomposition and photosynthetic activity in both the summer and winter or from man made sources such as discharge of wastes with high oxygen demands or cultural eutrophication. When reduced oxygen levels are combined with either high temperatures and/or high carbon dioxide concentrations and/or contaminants, fish are subjected to greater stress. Several reviews of the minimum oxygen requirements of freshwater fishes have been completed and may prove useful in evaluating field data (9, 10, 14, 16, 27, 39).

2) Lethal Temperatures. Temperature is most often a contributing factor in fish kills although industries and power generating stations sometimes discharge thermal effluents which are lethal to fish. Increases in temperature affect fish by increasing metabolic rate and oxygen demand, increasing the toxicity of substances, and by favouring growth of pathogens. Young life stages are most sensitive to lethal temperatures and abrupt temperature changes. Reviews on temperature preferences, selected, avoided, and lethal temperatures have been compiled for many species of fish (3, 6, 7, 8, 11, 18, 36).

3) Gas Bubble Disease. An excess of total dissolved gases may cause a condition in fish called gas bubble disease. Gas bubble disease is physically characterized by bubbles in the tail, fins, and mouth of fish (emphysema) and sometimes by eyes that are popped out from their sockets (exophthalmia). Death is caused by bubbles (emboli) in the blood stream which block blood flow through gills and tissues. The sum of partial pressures of all dissolved gases (CO_2, O_2, N_2, Ar) must be greater than ambient pressure and result in 110% saturation to

precipitate gas bubble disease in most fishes. Supersaturated water may originate from springs and wells under pressure, waterfalls, and the rapid heating of very cold water, reducing the solubility of gases and consequently increasing the saturation (2, 5, 26, 39, 40).

4) pH = -log $[H^+]$. Mass mortality of fish due to low pH waters has been observed in Norway (20, 22, 23). Although similar kills have not yet been reported in Ontario, the increasing frequency of acidic precipitation could eventually result in fish mortality after periods of heavy rain or snow melt, causing runoff of extremely low pH. Low pH waters can also originate from natural leaching and mining or industrial wastes. Acutely lethal, low pH's cause a failure of body salt regulation in fish, while chronically low pH levels may lead to reproductive failure or increased susceptibility to disease.

Alkalinity of water is a measure of the buffering capacity as determined by the presence of carbonates, bicarbonates, hydroxides and some less abundant bases. High pH can be acutely lethal. It may result naturally from algal blooms or leaching, or be due to sewage outfalls or agricultural runoff. A relatively high or low pH may not directly result in mortality but can increase the toxicity of other compounds present in the water. For example, ammonia is ten times as toxic at pH 8.0 as at pH 7.(5, 16, 22, 27, 34, 39, 40).

5) Fish Diseases and Parasites. Diseases can result in mass mortality in both hatchery and wild fish populations, although fish held in crowded culture conditions are more subject to mortality from disease. Fish diseases may be of bacterial, fungal or viral origin. All diseases are mediated by environmental factors, the physical state of the fish and exposure to contaminants. In addition to disease, parasites, alone or in combination with other environmental stresses, have also resulted in serious fish kills. The identification of fish mortality due to disease or parasitism is best accomplished by trained experts and proper preservation of biological samples is essential, (see Appendix C) (19, 33, 40).

6) Spawning-related mortalities. Mass mortality of some fish species after spawning (eg. alewife, smelt, trout-perch) is a fairly frequent occurrence. Post-spawning mortality of smelt has been reported regularly in Lake Erie. Massive mortalities of alewife in the Great Lakes are often recorded during the spring and summer months when the alewife congregate in the warmer shoreline waters of the Great Lakes in order to spawn. Although the cause of alewife mortalities has not been conclusively identified, it is believed that the die-offs result from the inability of the alewife to acclimate to rapidly fluctuating temperatures. Other species are also subject to post-spawning mortalities. The investigator should refer to a brief summary of the biology of the species to determine if this factor may have contributed to a fish kill, particularly in cases when only one species of fish is involved. (36).

7) Ammonia. Ammonia is a natural component of ground and surface waters but high levels can result from sewage, industrial and agricultural sources as well as from biological degradation. The unionized fraction of ammonia is most toxic and its proportion is dependent on water pH and temperature. Acutely lethal levels of ammonia may result in gill and other tissue damage, disturbed water balance and reduction in the number of red blood cells in fish. For long-term protection of all aquatic life, water should contain no more than .02 mg/l unionized ammonia (NH_3). Unionized ammonia levels above 0.2 mg/l are acutely lethal to some fish species (5, 12, 16, 18, 27, 29).

8) Chlorine. Chlorine is used in public water supplies as a disinfectant and industrially as a bleaching and antifouling agent. Free chlorine and chlorine combined with nitrogenous organic material (chloramines) are toxic to fish. Residual chlorine, the sum of free chlorine and chloramines, has been reported to kill salmonid fishes at a concentration of 10 µg/l in a period of several days. Salmonid fry are even more sensitive to residual chlorine. The provincial water quality objectives recommend a criterion of 2 µg/l to afford protection for all freshwater and marine aquatic life (4, 13, 16, 17, 27, 39).

9) Heavy Metals. Some heavy metals are biologically essential and others non-essential, but all are potentially harmful to most organisms at some level of exposure. Metals may be present naturally in a system, however, acutely lethal levels can generally be attributed to industrial discharges. The toxicity of heavy metals depends on the metal (As, Cd, Cr, Cu, Fe, Hg, Ni, Pb, Se, Si, Zn etc.), the species of fish, and the hardness of receiving waters. Two or more metals present in a system may act together and the resultant toxicity may be greater than the sum of individual toxicities (5, 6, 18, 24, 27, 28, 39).

10) Pesticides. Pesticides can be broadly categorized by use into insecticides, herbicides, fungicides, and piscicides. The aquatic toxicity of a particular pesticide depends on its chemical and physical properties, the nature of the receiving waters, and on the species affected. Organic pesticides which may persist in the environment, bioaccumulate, and resist degradation, are cited as potential chronic problems for aquatic life and human health. However, fish kills have been caused by acutely lethal levels of pesticides resulting from careless use or disposal near water systems or the application on the water of herbicides to control algae or rooted aquatic vegetation (1, 5, 16, 18, 21, 25, 27, 29, 30, 32, 39).

11) Algal Toxins. Toxins from blue green algae have been reported to cause the deaths of both farm animals and fish. The most noxious species is Microcystis, although toxins of Nodularia, Anabaena, Gloeotrichia and possibly Aphanizomenon have also been identified (31, 35). Occurrences of fish kills resulting from algal toxins are rare; oxygen depletion due to phytoplankton blooms is a more frequent cause.

Appendix B: Water chemistry sample information: specific parameters, sample containers, volume required and preservation techniques

Parameter	Container	Preservation Technique	Minimum Volume Required for each Parameter (ml)	Comment (Syn = Synonym)
Major Ions				
Alkalinity	Glass or Plastic	None	50	
Calcium	" "		40	
Chloride	" "		50	
Conductivity	" "		75	Syn = Specific Conductance
Hardness	" "		50	
Magnesium	" "		40	
Potassium	Plastic or Glass		40	
Silicates - Reactive	Plastic only		50	Syn = Silica
Sodium	Glass or Plastic		40	
Sulphate	" "		50	
Nutrients				
Ammonia Nitrogen (Filtered)	Glass or Plastic (polystyrene not linear polyethlene)	Freeze or Refrigerate	75	Syn = Nitrogen - Ammonia
Nitrate Nitrogen (Filtered)				Syn = Nitrogen - Nitrate
Nitrite Nitrogen (Filtered)				
Orthophosphate				Syn = Phosphorus - Filtered Reactive
Nutrient - Total Kjeldahl				
Phosphorus - Total				Syn = Total Phosphorus

Appendix B (continued)

Parameter	Container	Preservation Technique	Minimum Volume Required for each Parameter (ml)	Comment (Syn = Synonym)
Metals				
Aluminum Barium Cadmium Chromium Cobalt Copper Iron Lead Lithium Manganese Molybdenum Nickel Selenium Silver Strontium Titanium Uranium Vanadium Zinc	Plastic or Glass[2]	HNO_3 to pH of <2 (approximately 20 drops per litre bottle)[1]	100 - unless preconcentration required	
Arsenic	Plastic or Glass[2]	None	50	
Boron	Plastic only	None	100	
Mercury	Glass only	HNO_3 to pH of 1 + $KMnO_4$ to maintain slight purple colour/176 ml bottle	176[3]	

[1]Nitric acid preservative should be added AFTER the sample is placed in the bottle.

[2]Acid washed plastic containers are recommended for ultra-trace analysis; N.B. foil cap liners of glass bottles used for routine samples may cause contamination, and plastic lined caps are recommended.

[3]A special 176 ml sample bottle similar to the microbial analysis type is usually provided for Hg samples.

Appendix B: (continued)

Parameter	Container	Preservation Technique	Minimum Volume Required for each Parameter (ml)	Comment (Syn = Synonym)
Organic Parameters				
Anionic Detergents	Glass	Refrigerate	100	Syn = L.A.S., Linear Alkyl Sulfonates, methylene Blue Active Substances, Detergents.
Biochemical Oxygen Demand	Glass		500	Syn = BOD_5
Carbon - Total Organic	Glass or Plastic		50	Syn = TOC
Carbon - Inorganic	Glass or Plastic		50	Syn = IC
Carbon Dioxide	Special*		*	Syn = Free CO_2. Special sampling required.
Chemical Oxygen Demand	Glass		25	Syn = COD
Colour - Apparent	Glass		75	Syn = Apparent Colour
Pesticides	Glass only		900	Syn = Chlorinated Hydrocarbons
Petroleum Hydrocarbons	Glass only		900	Syn = Hydrocarbons
Solvent Extractables	Glass		900	Syn = Ether solubles
Tannins and Lignins	Glass		200	
Volatile Acids	Glass		25	

*CO_2 samples are to be carefully transferred from the sampling device into the bottom of a leak-proof glass stoppered container so as to prevent splashing (syphon); after copious overflow the bottle must be stoppered so that no air bubbles are present in the container and rushed to a laboratory.

Appendix B: (continued)

Parameters	Container	Preservation Technique	Minimum Volume Required for each Parameter (ml)	Comment (Syn = Synonym)
Other Parameters				
Acidity	Glass or Plastic	None	50	
Chlorophyll *a* and *b*	Field Filtration Required	5 drops of 1.0% $MgCO_3$ per litre of sample prior to filtration	500	Contact Water Quality Section if there are any questions. When chlorophyll analyses are desired, a duplicate phytoplankton sample should be collected, preserved, and submitted (see: Biological Sampling - Phytoplankton).
Cyanide	Glass or Plastic	Add NaOH pellets to pH >11	500	
Fluoride	Glass or Plastic	None	50	
Particle Size Analysis (Sediment)	Glass or Plastic	None	100 g.	
pH	Glass or Plastic	None	25	
Phenolics-Reactive	Special	Provided	150[1]	Obtain special bottle with preservative
Plasticity (Sediment)	Glass or Plastic	None	100 g	
Resins & Fatty Acids	Glass only	Add concentrated H_2SO_4 or HCl to pH 3.	900	One bottle should be submitted for this test exclusively, labelled Resin Acids. Syn = Fatty Acids
Settleability	Glass	None	900	
Sludge Volume Index	None	None	None	Calculated Parameter

[1] A special 176 ml sample bottle similar to the microbial type (with preservative added) is usually provided for samples requiring analysis for phenolics. A culture tube sample container with preservative is also available.

Appendix C: Handling specimens for histopathological examination: collection, preservation, shipping

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1. Fish tissue must be fixed as soon after death as possible.

2. Tissue for histopathological examination should not be frozen. (Keep it iced, or refrigerated if fixative is not at hand).

3. Fixative: use 10% formalin (i.e. 10 ml. commercial formalin in 90 ml. water)

4. The volume of fixative should be at least ten times the volume of the specimen. (Do not squeeze large specimens into small jars).

5. Use glass or other corrosion-resistant containers for formalin.

6. Small fish - up to 4 or 5 inches in length - may be fixed whole. Slit open the muscle along the ventral surface and remove the covering muscle on one side of the abdomen in the larger fish, to allow fixative to reach internal organs quickly. Keep fish flat--do not bend or twist while fixing.

7. If lesions are large, eg. tumors, cut a slice off one end approximately 1/4" thick for rapid fixing, cut the rest in halves or quarters, fix and send all tissue. If possible take a photograph of the lesion before it is cut, and in situ. Include some adjacent normal tissue with the lesion, when fixing.

8. For mailing - remove tissue from jar of formalin after 48 hours (for thin tissue), or longer. Place the tissue, wrapped in cheesecloth or paper towelling and moistened with enough 10% formalin to keep it moist, in a tightly sealed plastic bag. Pack the tissue so that it will not be crushed in the mail.

   Include with the specimen your name and address, your specimen identification number and species of fish. Supply any pertinent information that is available, eg.:

   a) location, date, and method of catch

   b) gross description of the lesion-where found etc.

   c) size (length/weight), sex, and age of fish

   d) evidence of abnormal behaviour

   e) whether wild or cultivated fish

   f) the number of fish with similar lesions

NOTE:

Bacteriological or virological identification cannot be undertaken on formalin-fixed tissue. However, it is possible to observe cellular changes which indicate infection by microorganisms.

For isolation of bacteria or viruses it is important that sick fish be killed, wrapped individually in waxed paper, placed in plastic bags and quick-frozen at once. The tissue must remain frozen until it reaches a diagnostic laboratory.

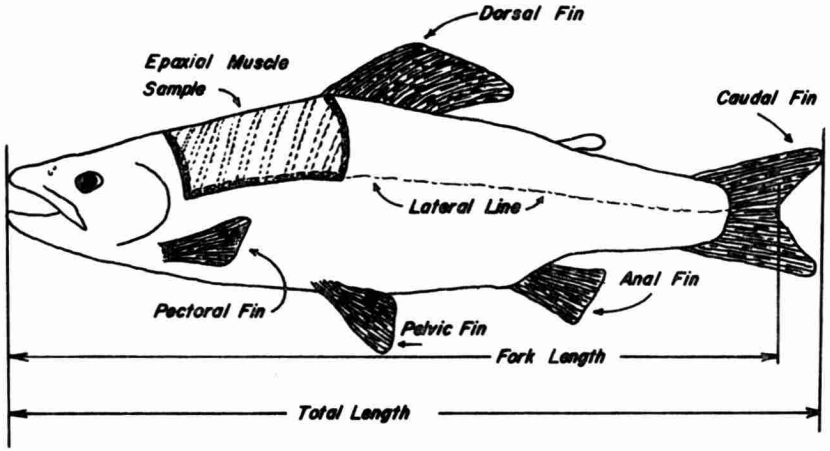
Surface contaminants and other bacteria commonly in the gastro-intestinal tract multiply rapidly in unfrozen tissue. Viruses may be destroyed in unfrozen tissue. Tissues must be taken immediately after death.

9) Arrangements for histopathological examination of fish specimens should be made through the Toxicity Unit Staff, Resources Road, Rexdale, (phone: (416) 248-3011).

Appendix D: Sampling fish muscle for residue analysis after long-term exposure to a contaminant.

When fish are exposed to certain contaminants for extended periods of time it is sometimes possible to analyze fish muscle samples for residues of the contaminant (eg. mercury and PCB's). If muscle residue analysis is desired, the investigator should dissect a sample of fish muscle from above the lateral line (epaxial muscle) of a dead specimen (see diagram opposite) and preserve it for analysis. For metal determinations the muscle is placed in small plastic bags and then frozen. Samples collected for PCB, pesticide or organic trace contaminant analysis, are first wrapped in solvent washed aluminum foil and then frozen. All samples must be properly labelled.

N.B. In the case of a fish kill it is less likely that the fish exposed to a lethal concentration of a contaminant will have accumulated detectable muscle levels. However, it is sometimes possible to detect contaminant residues in other parts of the body. See text on "Sampling for tissue residue analysis" for further instructions.


Dorsal Fin
Epaxial Muscle Sample
Caudal Fin
Lateral Line
Anal Fin
Pectoral Fin
Pelvic Fin
Fork Length
Total Length

Appendix E: Where to report a fish kill - Ministry of the Environment and Ministry of Natural Resources regional and district offices

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Northwestern and North Central Regions
Ministry of the Environment

Thunder Bay Regional Office	(807) 475-1215
Thunder Bay District Office	(807) 475-1305
Kenora District Office	(807) 468-5578

Ministry of Natural Resources

Dryden District Office	(807) 223-3341
Fort Frances District Office	(807) 274-5337
Ignace District Office	(807) 934-2233
Kenora District Office	(807) 468-9841
Red Lake District Office	(807) 727-2531
Sioux Lookout District Office	(807) 737-1140
Atikokan District Office	(807) 597-6971
Geraldton District Office	(807) 854-1030
Nipigon District Office	(807) 887-2120
Terrace Bay District Office	(807) 825-3205
Thunder Bay District Office	(807) 475-1511
White River District Office	(807) 822-2250

Northern and Northeastern Regions
Ministry of the Environment

Sudbury Regional Office	(705) 522-8282
Sudbury District Office	(705) 522-8282
Timmins District Office	(705) 264-9474
Sault Ste Marie District Office	(705) 949-4640
North Bay District Office	(705) 476-1001
Parry Sound District Office	(705) 746-2139

Ministry of Natural Resources

Chapleau District Office	(705) 864-1710
Cochrane District Office	(705) 272-4365
Gogama District Office	(705) 894-2000
Hearst District Office	(705) 362-4346
Kapuskasing District Office	(705) 335-6191
Kirkland Lake District Office	(705) 642-3222
Moosonee District Office	(705) 336-2987
Timmins District Office	(705) 264-1262
Blind River District Office	(705) 356-2234
Espanola District Office	(705) 869-1330
North Bay District Office	(705) 474-5550
Sault Ste Marie District Office	(705) 949-1231
Sudbury District Office	(705) 522-7823
Temagami District Office	(705) 569-3622
Wawa District Office	(705) 856-2396

Central and Algonquin Regions
Ministry of the Environment

Central Regional Office	(416) 424-3000
South Peel Area System	(416) 270-1451
Barrie District Office	(705) 726-1730
Muskoka-Haliburton District Office	(705) 687-3408
Peterborough District Office	(705) 743-2972
Toronto District Office	(416) 424-3000
Halton-Peel District Office	(416) 844-5747
Huntsville Sub-Office	(705) 789-2386

Ministry of Natural Resources

Cambridge District Office	(519) 658-9356
Niagara District Office	(416) 892-2656
Lindsay District Office	(705) 324-6121
Maple District Office	(416) 832-2261
Huronia District Office	(705) 728-2900
Bancroft District Office	(613) 332-3940
Minden District Office	(705) 286-1521
Parry Sound District Office	(705) 746-2141
Pembroke District Office	(613) 732-3661
Algonquin Park District Office	(705) 637-2780
Bracebridge District Office	(705) 645-5244

Southern and West Central Regions
Ministry of the Environment

London Regional Office	(519) 681-3600
Windsor District Office	(519) 254-5129
Sarnia District Office	(519) 336-4030
Owen Sound District Office	(519) 371-2901
Chatham Sub-District Office	(519) 352-5107
Hamilton Regional Office	(416) 561-7410
Cambridge District Office	(519) 623-2080
Welland District Office	(416) 735-0431
Simcoe Sub-office	(519) 426-1940

Ministry of Natural Resources

Aylmer District Office	(519) 773-9241
Chatham District Office	(519) 354-7340
Owen Sound District Office	(519) 376-3860
Simcoe District Office	(519) 426-7650
Wingham District Office	(519) 357-3131

Southeastern and Eastern Regions
Ministry of the Environment

Kingston Regional Office	(613) 549-4000
Ottawa District Office	(613) 521-3450
Cornwall District Office	(613) 933-7402
Belleville District Office	(613) 962-9208
Pembroke District Office	(613) 732-3643

Ministry of Natural Resources

Brockville District Office	(613) 342-8524
Cornwall District Office	(613) 933-1774
Lanark District Office	(613) 259-2108
Napanee District Office	(613) 354-2173
Ottawa District Office	(613) 822-2525
Tweed District Office	(613) 478-2330

Field Notes

Field Notes

Field Notes

Field Notes